

WHAT IS CLAIMED IS:

1. A method of separating a compound of interest from a mixture, the method comprising the steps of:

(a) providing a mixture containing a compound of interest, the compound of interest having an expected mass;

(b) subjecting a portion of the mixture to a separation using an analytical HPLC column to produce an eluate stream;

(c) analyzing the eluate stream using a mass spectrometer to determine a retention time of the compound of interest on the analytical HPLC column;

(d) predicting an elution time for the compound of interest from a preparative scale HPLC column by:

(1) predicting a retention time of the compound of interest from the preparative scale HPLC column using a predetermined correlation function between the analytical HPLC column and the preparative scale HPLC column along with the determined retention time of the compound on the analytical HPLC column; and

(2) selecting a window of time around the predicted retention time within which the compound is expected to elute;

(e) subjecting all or a portion of the remaining mixture to a preparative scale HPLC system comprising a preparative scale HPLC column, an HPLC compatible detector, and a fraction collector; and

(f) collecting at least a portion of the compound of interest using the fraction collector, the fraction collector being activated upon detection of a peak by the HPLC compatible detector within the predicted elution time.

2. The method of claim 1 wherein the HPLC detector is selected from the group consisting of UV detector, ELSD, refractive index detector, and chemiluminescent nitrogen detector.

3. The method of claim 1 wherein data collected from the mass spectrometer consists essentially of the expected mass of the compound of interest and the retention time of the compound on the analytical HPLC column.

4. The method of claim 1 wherein a UV chromatogram is not generated from the separation using the analytical HPLC column.

5. A method of separating a compound of interest from a mixture, the method comprising the steps of:

- (a) providing a mixture containing a compound of interest, the compound of interest having an expected mass;
- (b) subjecting a portion of the mixture to a separation using an analytical HPLC column to produce an eluate stream;
- (c) analyzing the eluate stream using a mass spectrometer to determine a retention time of the compound of interest on the analytical HPLC column;
- (d) predicting an elution time for the compound of interest from a preparative scale HPLC column using the determined retention time of the compound of interest on the analytical HPLC column;
- (e) subjecting all or a portion of the remaining mixture to a preparative scale HPLC system comprising a preparative scale HPLC column; and
- (f) collecting at least a portion of the compound of interest using the predicted elution time.

6. The method of claim 5 wherein the step of predicting an elution time for the compound of interest comprises:

(1) predicting a retention time of the compound of interest from the preparative scale HPLC column using a predetermined correlation function between the analytical HPLC column and the preparative scale HPLC column along with the determined retention time of the compound on the analytical HPLC column; and

(2) selecting a window of time around the predicted retention time within which the compound is expected to elute.

(7) The method of claim 5 wherein an artificial neural network is used to predict the elution time in step (d).

(8) The method of claim 5 wherein a dynamic correlation function is used to predict the elution time in step (d).

9. The method of claim 5 wherein the preparative scale HPLC system includes an HPLC compatible detector.

(10) The method of claim 9 wherein the detector is selected from the group consisting of UV detector, ELSD, refractive index detector, and chemiluminescent nitrogen detector.

(11) The method of claim 5 wherein data collected from the mass spectrometer consists essentially of the expected mass of the compound of interest and the retention time of the compound on the analytical HPLC column.

12. The method of claim 5 wherein a UV chromatogram is not generated from the separation using the analytical HPLC column.

13. The method of claim 6 wherein a UV chromatogram is not generated from the separation using the analytical HPLC column.

14. The method of claim 9 wherein the HPLC system includes a fraction collector that collects at least a portion of the compound of interest, the fraction collector being activated upon detection of a peak by the HPLC compatible detector within the predicted elution time.

15. The method of claim 5 wherein the preparative scale HPLC system does not include an HPLC compatible detector.

16. The method of claim 5 wherein the collection of step (f) is performed without the use of an HPLC compatible detector.

17. A method of separating compounds of interest present in a plurality of reaction product mixtures, the method comprising the steps of:

- (a) providing a plurality of reaction product mixtures, each mixture expected to contain a compound of interest having an expected mass;
- (b) separately subjecting a portion of each reaction product mixture to a separation using an analytical HPLC column to produce a plurality of eluate streams;
- (c) analyzing each eluate stream using a mass spectrometer to verify that the eluate stream contains a compound with an expected mass and to determine a retention time of each compound with an expected mass on the analytical HPLC column;
- (d) predicting an elution time for each compound with an expected mass from a preparative scale HPLC column using the determined retention time for each compound with an expected mass on the analytical HPLC column;



(e) separately subjecting all or a portion of each remaining mixture verified to contain a compound with the expected mass to a preparative scale HPLC system comprising a preparative scale HPLC column and a fraction collector; and

(f) separately collecting at least a portion of each compound verified to be present with the fraction collector using the predicted elution time for each compound.

(18) The method of claim 17 wherein at least two eluate streams produced in step (b) are combined for analysis in step (c) in a common mass spectrometer, the at least two eluate streams being chosen such that the expected compounds of interest in the streams have different expected masses that are distinguishable by mass spectrometry.

(19) The method of claim 17 wherein at least two eluate streams produced in step (b) are combined for analysis in step (c) in a common mass spectrometer, at least one of the eluate streams being mechanically coded such that the expected compounds of interest in the streams are distinguishable by mass spectrometry.

(20) The method of claim 17 wherein the step of predicting an elution time for each compound comprises:

(1) predicting a retention time of each compound with an expected mass on the preparative scale HPLC column using a predetermined correlation function between the analytical HPLC column and the preparative scale HPLC column along with the determined retention time of each compound on the analytical HPLC column; and

(2) selecting a window of time around each predicted retention time within which each compound with an expected mass is expected to elute.

(21) The method of claim 17 wherein an artificial neural network is used to predict the elution time for each compound in step (d).

22. The method of claim 17 further comprising determining the purity of the compound of interest immediately following the separation using the analytical HPLC column.

(23) The method of claim 17 wherein the preparative scale HPLC system includes an HPLC compatible detector.

(24) The method of claim 23 wherein the detector is selected from the group consisting of UV detector, ELSD, refractive index detector, and chemiluminescent nitrogen detector.

25. The method of claim 17 wherein a UV chromatogram is not generated from the separation using the analytical HPLC column.

(26) The method of claim 18 wherein the step of predicting an elution time for each compound of interest comprises:

(1) predicting a retention time of each compound with an expected mass on the preparative scale HPLC column using a predetermined correlation function between the analytical HPLC column and the preparative scale HPLC column along with the determined retention time of each compound on the analytical HPLC column; and

(2) selecting a window of time around each predicted retention time within which each compound with an expected mass is expected to elute.

27. The method of claim 26 wherein a UV chromatogram is not generated from the separation using the analytical HPLC column.

(28) The method of claim 27 wherein the preparative scale HPLC system includes an HPLC compatible detector.

(29) The method of claim 28 wherein the preparative scale HPLC system includes a fraction collector that collects at least a portion of the compound of interest, the fraction collector being activated upon detection of a peak by the HPLC compatible detector within the predicted elution time.

30. A method of separating a compound of interest from a mixture, the method comprising the steps of:

- (a) providing a mixture containing a compound of interest;
- (b) subjecting a portion of the mixture to a separation using thin layer chromatography to determine an R_f value for the compound of interest;
- (c) predicting an elution time of the compound of interest on a preparative scale HPLC column using the determined R_f value for the compound of interest;
- (d) subjecting all or a portion of the remaining mixture to a preparative scale HPLC system comprising a preparative scale HPLC column; and
- (e) collecting at least a portion of the compound of interest using the predicted elution time.

31. The method of claim 30 wherein the step of predicting an elution time for the compound of interest comprises:

- (1) predicting a retention time of the compound of interest from the preparative scale HPLC column using a predetermined correlation function between

Rf value from the TLC and retention time on the preparative scale HPLC column along with the determined Rf value of the compound of interest; and

(2) selecting a window of time around the predicted retention time within which the compound is expected to elute.

32. The method of claim 30 wherein step (b) comprises:

(1) subjecting a portion of the mixture to a separation using thin layer chromatography to produce one or more spots or zones;

(2) analyzing the one or more spots or zones using a mass spectrometer to determine the spot or zone containing the compound of interest; and

(3) determining an Rf value for the compound of interest.

33. The method of claim 30 wherein an artificial neural network is used to predict the elution time in step (c).

34. The method of claim 30 wherein a dynamic correlation function is used to predict the elution time in step (c).